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Effect of tyrosol in *Candida* species biofilm development

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Candida spp. are the most common agents of opportunistic mycoses and are often associated with biofilms. The production of diffusible alcohol molecules allows the modulation of microbial physiological functions and probably offers a strategy for communication between *Candida* spp.. Tyrosol has been shown to be a quorum-sensing signal in *Candida albicans* that is accumulated in planktonic and biofilm supernatants. Although tyrosol accelerates *C. albicans* germ tube formation under inducing conditions, the effect of tyrosol on biofilm formation is not clear. Additionally, to date it is not known whether tyrosol affects biofilm development of Non - *Candida albicans Candida* (NCAC) spp..

In this sense, this work aimed to examine the effect of a commercial formulation of tyrosol on different stages of NCAC spp. biofilm maturation, as an indicator of a role for this compound in biofilm development.

Using a rapid and robust method (96-well plate model), the effect of tyrosol (10, 100 and 1000 µM) on *C. dubliniensis* CBS 7987, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750 biofilm formation was examined at different stages. *C. albicans* CECT 1472 was used as a control. Standardized cell suspensions (200 µl of 1×10^6 cells/ml in RPMI) were inoculated into microtitre plates and incubated for 3 and 48 h at 37°C, 130 rpm. At these time points the medium was removed and sessile cells were washed with ultrapure sterile water. Tyrosol was then added to *Candida* spp. sessile cells and the plates were incubated at 37°C for additional 24 h. Biofilm cells mitochondrial activity was evaluated by the XTT reduction assay and biofilm total biomass by crystal violet assay.

The results of these experiments showed that tyrosol did not exhibit major effects on *C. albicans* and *C. dubliniensis* biofilm development. But, in contrast, tyrosol (10 and 100 µM) induced a significant reduction in the mitochondrial activity of *C. parapsilosis* sessile population and mature biofilms during subsequent development. The highest reduction levels of absorbance at 490 nm, compared with the control, were observed in mature biofilm cells treated for 24h with 10 and 100 µM tyrosol. Furthermore, addition of tyrosol (100 and 1000 µM) to *C. tropicalis* adhered cells population led to a significant decrease in biofilm cells mitochondrial activity during further development, as indicated by the lower levels of XTT readings compared with control biofilms. Under the conditions used in this study no changes were detected in total biomass of *Candida* spp. sessile cells treated with tyrosol.

These results show that tyrosol regulates *C. parapsilosis* and *C. tropicalis* biofilm cells mitochondrial activity, suggesting a role of this alcohol in the communication between *Candida* spp..

Keywords Candida spp.; biofilm; tyrosol